Some Properties of DNA from the Prosobranch Gastropod *Tegula funebralis*

BY

PAUL C. SCHROEDER

AND

CHARLES F. CLELAND

Department of Biological Sciences, Stanford University

This communication reports the isolation and partial characterization of deoxyribonucleic acid (DNA) from the testis of the marine prosobranch *Tegula funebralis*. Estimates of the DNA nucleotide composition from chemical analysis, buoyant density (Schildkraut, Marmur & Doty, 1962), and thermal denaturation temperature (Marmur & Doty, 1962) are in good agreement. Of additional interest is the presence in digests of *Tegula* DNA of at least one unidentified chromatographic component, presumably an unusual nucleotide, which represents 3 to 6% of the total nucleotide content.

MATERIALS AND METHODS

Specimens of *Tegula (Chlorostoma) funebralis* (A. Adams, 1854), collected from high tidepools in the intertidal area at Arroyo de los Frijoles in San Mateo County, California on February 16, 1963, were stored at 13° C. and dissected in the cold within 72 hours of collection. After the shells had been cracked in a vise, the testis of each male was dissected away from the closely adjacent hepatopancreas; minute portions of this organ were unavoidably included with the sample. Dissection of approximately fifty males yielded 7.1 grams of testicular tissue, which appeared on microscopic examination to consist largely of mature sperm.

From this tissue DNA was isolated by the method of Kay, Simmons & Dounce (1952), except that the product was at no time subjected to air drying. The final product was dissolved in 0.02M NaCl and stored at 4° C.

Deoxypentose was determined by the method of Dische (1930) and pentose by the method of Mejbaum (1939). Protein estimation was carried out according to Lowry *et al.* (1951), using purified bovine serum albumin as a standard.

To determine the nucleotide composition of the DNA a sample corresponding to 0.9 micromole of nucleotide phosphate was degraded completely to 5′— mononucleotides by sequential treatment with bovine pancreatic DNase (Worthington Biochemical Corporation) and purified snake venom diesterase (Koerner & Sinsheimer, 1957). Following chromatography of the digest on Whatman No. 1 paper in an ammonium sulfate—sodium acetate—isopropanol solvent (Markham & Smith, 1952), spots corresponding to the nucleotide components were located with a UV lamp, cut out and eluted in 0.01M potassium phosphate buffer, pH 7. Using published extinction coefficients (Beaven, Holiday & Johnson, 1955), the nucleotides were identified and quantitatively estimated from measurements of optical density at several wave lengths with a Zeiss spectrophotometer. Recovery of chromatographed material was between 80 and 93%.

For buoyant density determination, samples of *Tegula* DNA were centrifuged with a reference DNA of known density in concentrated solutions of cesium chloride for 16 to 20 hours at 44,770 rpm in a Spinco Model E analytical ultracentrifuge equipped with a UV optical system (Meselson, Stahl & Vinograd, 1957; Schildkraut, Marmur & Doty, 1962). Buoyant density values were calculated from tracings made with a Joyce-Loebl double beam microdensitometer of UV photographs taken of the cells at equilibrium. As reference DNAs, the synthetic deoxyadenylate — deoxythymidylate copolymer (dAT, Schachman *et al.*, 1960; $\rho = 1.679$ gm cm^{-3}) and N^{15}-labelled *Pseudomonas aeruginosa* DNA ($\rho = 1.742$ gm cm^{-3}), kindly provided by Dr. Schildkraut, were used.

To determine the thermal denaturation temperature (Marmur & Doty, 1959) changes in optical density at 260 mμ of DNA solutions in stoppered quartz cuvettes were followed in a Zeiss PMQ spectrophotometer equipped to allow measurement of the cell temperature and regulation of the temperature to within a half degree. Determinations were carried out in a solution of 0.075M NaCl and 0.0075M Na citrate; corrections for ionic strength differences were made according to Inman & Baldwin (unpublished data) to facilitate comparison with values obtained at other salt concentrations.

RESULTS

Purity: Chemical analyses of the isolated *Tegula* DNA showed little contamination with protein or with RNA. Protein could be excluded to a level of less than 0.1% by weight; the ratio of purine pentose to deoxypentose was 0.003.

Chemical analysis of nucleotide composition: Whereas control analyses of calf thymus DNA gave only the four commonly occurring deoxyribonucleotides in amounts expected from published data (Beaven, Holiday & Johnson, 1955), chromatograms of *Tegula* DNA digests consistently showed five distinct UV-absorbing spots. Four of these, in the order in which they chromatographed, could be identified as the 5′—deoxyribonucleotides of adenine (slowest moving), guanine, thymine and cytosine (Table I). The fifth component, which migrated ahead of deoxycytidylate, gave absorbency ratios which did not appear to correspond with published values for any of the "rare" nucleotides previously identified in DNA from other sources. Its chromatographic behavior and absorbency ratios suggested that it might be a deoxycytidylate derivative; however, authentic samples of 5′-5-methyl- and 5′-5-hydroxymethyl deoxycytidylate did not cochromatograph with the unknown component.

A sixth component, present in smaller amount and imperfectly resolved from deoxyadenylate was also observed on the chromatograms. The identity of these components remains undetermined.

Table 1

UV Absorbancy Ratios and Quantitative Estimation of *Tegula* DNA Nucleotides

Nucleotide	Absorbancy Ratios 250 mμ/260mμ	280 mμ/260mμ	Quantity Present μ Moles
Experiment I			
dAMP	.75	.14	.19
dGMP	1.12	.68	.13
dTMP	.72	.75	.24
dCMP	.79	.98	.11
Unknown	.87	.67	.02-.04[1]
Experiment II			
dAMP	.77	.07	.19
dGMP	1.12	.68	.14
dTMP	.62	.65	.31
dCMP	.79	.98	.13
Unknown	.81	.68	.02-.04[1]

[1]Assuming a molar extinction coefficient at 260 mμ of 7500 to 15000.

Buoyant Density in Cesium Chloride: Equilibrium density gradient sedimentation of *Tegula* DNA in CsCl showed a major symmetrical band at a buoyant density of 1.697 gm. cm. $^{-3}$. In runs using the N^{15} *Pseudomonas aeruginosa* DNA as a reference, a small "satellite" component, corresponding to approximately 1% of the total DNA, could also be detected at a density of 1.682 gm. cm. $^{-3}$. Similar low density components have been described previously by Sueoka (1961) in DNA from *Cancer irroratus* T. Say, 1817 and *Cancer borealis* W. Stimpson, 1859. They have been shown to consist almost entirely of deoxyadenylate and deoxythymidylate residues (Sueoka & Cheng, 1962).

Thermal Denaturation Temperature: Measurement of optical density of a solution of *Tegula* DNA as a function of increasing temperature showed the sharp hyperchromic transition characteristic of native DNA (Marmur & Doty, 1959). The transition occurred over a range of approximately 14° C, with a midpoint (T_m) of 78.5° C. This figure corresponds to a value of 84° C at the ionic strength employed by Marmur & Doty (1962).

Table 2

Comparison of Estimations of *Tegula* DNA Nucleotide Composition

Method	Guanine + Cytosine Content
Chemical Analysis	38%[1]
Buoyant Density	38%
Thermal Denaturation Temperature	36%

[1] Assuming that the unknown component is a derivative of deoxycytidylate.

CONCLUSIONS

From the above determinations, the nucleotide composition of *Tegula* DNA can be estimated (Table II) using the established empirical relationships of guanine-cytosine (GC) content with buoyant density (Schildkraut, Marmur & Doty, 1962) and thermal denaturation temperature (Marmur & Doty, 1962). The values so obtained are in quite good agreement with the results obtained by chemical analysis. It may be concluded that the GC content of *Tegula* DNA is approximately 38%. This value is similar to that reported for *Anodonta sp.* (37%), *Chlamys (Pecten) islandica* (Müller, 1776) (40%) and *Patella sp.* (36%) (Antonov & Belozerskii, 1961) and for *Mercenaria (Venus) mercenaria* (Linnaeus, 1758) (37 %)

(Sueoka, 1961). All of these contrast with the value of 47% found by Antonov & Belozerskii (1961) for DNA from muscular tissue from *Buccinum undatum* Linnaeus, 1761.

The apparent presence of unusual nucleotides, as well as a low density "satellite" component in the DNA from *Tegula funebralis* makes it an interesting object for further study.

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